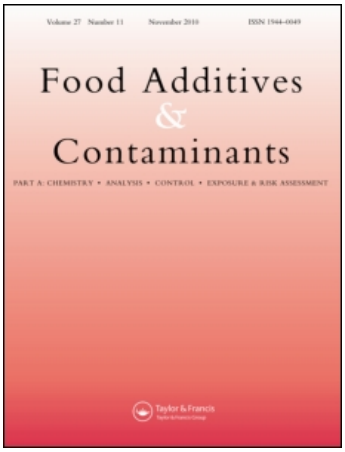

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Routine approach to qualitatively screening 300 pesticides and quantification of those frequently detected in fruit and vegetables using liquid chromatography tandem mass spectrometry (LC-MS/MS)

B. Kmellár[a]; L. Abrankó[a]; P. Fodor[a]; S. J. Lehotay[b]
[a] Department of Applied Chemistry, Faculty of Food Science, Corvinus University of Budapest, Budapest, Hungary [b] US Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, Wyndmoor, PA 19038, USA

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B. Kmellár[a]*, L. Abrankó[a], P. Fodor[a] and S.J. Lehotay[b]

[a]*Department of Applied Chemistry, Faculty of Food Science, Corvinus University of Budapest, 1118 Villányi Street 29–43, Budapest, Hungary;* [b]*US Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA*

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This paper describes an efficient and effective analytical scheme to first screen for 300 pesticides in fruit and vegetables samples using liquid chromatography tandem mass spectrometry (LC-MS/MS) with a commercially enhanced product ion method. Then presumed positive extracts are analysed using a quantitative and confirmatory LC-MS/MS method optimized for 55 pesticides. A quick, easy, cheap, effective, rugged, and safe (QuEChERS) method with acetate buffering (AOAC Official Method 2007.01) was used for sample preparation, which has been previously shown to yield high-quality results for hundreds of pesticide residues in foods. The advantages and disadvantages of both the qualitative screening and quantitative/confirmatory methods and their combination are critically discussed. No false-negatives for the 55 pesticides occurred above 10 ng g^{-1} for extracts analysed by both LC-MS/MS methods, and the no false-positives were encountered from the screening analysis (after analyst review) because all presumptive identifications were confirmed in the second analysis. The monitoring scheme was applied during a one-year period on 200 fruit and vegetable samples from Hungarian markets. No pesticide residues were found in half the samples, and twelve violations of European maximum residue limits were detected.

Keywords: liquid chromatography/mass spectrometry (LC/MS); pesticide residues; pesticides; fruit; mushrooms; vegetables

Introduction

Plant protection products (more commonly known as pesticides) are widely used in agriculture to increase the yield, improve the quality, and extend the storage life of food crops. The pesticides must undergo extensive efficacy, environmental, and toxicological testing to be registered by governments for legal use in specified applications. The applied chemicals and/or their degradation products may remain as residues in the agricultural products, which becomes a concern for human exposure. Therefore, maximum residue levels (MRLs) (or 'tolerances' in the United States) that limit the types and amounts of residues that can be legally present on foods are set by regulatory bodies worldwide. In Europe, European Union Council Directive 91/414/EEC (European Commission 1991) describes the regulatory framework by which MRLs are set. If farmers apply the pesticides properly on crops for which the pesticides have been registered, and appropriate harvest intervals are given, then it is very unlikely that regulatory limits will be exceeded. Unfortunately, not all farmers follow legal practices, and due to the tremendous number of pesticides and crops in production, there is a need for routine multiresidue pesticide monitoring using methods with a wide analytical scope (Fernández-Alba and García-Reyes 2008).

The most common techniques in modern multiresidue target pesticide analysis are gas chromatography and liquid chromatography coupled to mass spectrometry (GC-MS, LC-MS) and/or tandem mass spectrometry (GC-MS/MS, LC-MS/MS) with triple quadrupole mass analysers. The numerous methods available for pesticide analysis show the importance of this application and rapid pace of developments in analytical chemistry. For example, Agüera et al. (2000) described a method for the measurement of only ten organophosphorus and organochlorine pesticides by GC-MS, but over the past decade, the number of pesticides typically included in methods has increased dramatically. Reports in the literature on LC-MS/MS described the increasingly wider scope analysis of 19 (Granby et al. 2004), 32 (Pozo et al. 2007), 52 (Hernández et al. 2006), 74 (Ortelli et al. 2004), 82

*Corresponding author. Email: bela.kmellar@uni-corvinus.hu
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(Banerjee et al. 2007), 160 (Kmellár et al. 2008) or 169 (Pizzutti et al. 2007) pesticides and/or degradation products in fruit and vegetable samples.

The sample preparation techniques have also advanced to complement the analytical techniques depending on the types of analytes and matrices monitored. The 'quick, easy, cheap, effective, rugged, and safe' (QuEChERS) method for pesticide residues in foods (Anastassiades et al. 2003; Lehotay, De Kok et al. 2005; Lehotay, Mastovská et al. 2005; Lehotay 2007; European Committee of Standardization (CEN) 2008) is an example of a method that takes advantage of the powerful features of nearly universal selectivity and high sensitivity of modern GC- and LC-MS(/MS) instruments. The QuEChERS approach has been extensively validated for hundreds of pesticide residues in many types of foods, and has become Association of Analytical Communities (AOAC) Official Method 2007.01 (Lehotay 2007) and CEN (2008).

Triple quadrupole MS/MS instruments are mainly applicable for sensitive and selective quantitative measurements and the identification of known, targeted analytes in selected or multiple-reaction monitoring (SRM or MRM) mode. Recently, many new software and hardware capabilities have been introduced from MS/MS manufacturers to allow faster analysis times, lower detection limits, more ion transitions per given time, fast positive/negative switching, and beneficial data-handling features. One of the companies, Applied Biosystems (Foster City, CA, USA), introduced an enhanced product ion (EPI) feature in MRM mode for their linear ion-trap products using information-dependent acquisition (known as data-dependent scanning by other manufacturers) and MS/MS spectral libraries (Applied Biosystems 2005). Such products should be independently evaluated in real-world applications to assess their capabilities and limitations.

One of the major problems in LC-MS/MS pesticide analysis due to the typical use of electrospray ionization (ESI) is caused by matrix effects. Quantitative results in LC-MS/MS cannot be trusted unless matrix effects have been assessed and compensated for if they are found to occur (Granby et al. 2004; Niessen et al. 2006; Kruve et al. 2008). The best approach to compensate for matrix effects entails the use of isotopically labelled internal standards for each analyte, but this is not feasible in multiresidue analysis of so many pesticides. Therefore, the most common approach is to use matrix-matched calibration standards. However, it can be difficult to find a blank matrix from which to prepare the calibration standards, and compensation from one sample to another (even for the same matrix) may not be the same. A method of standard additions in the sample extract may be an alternative approach.

The aim of this study was to assess in real-world practice the combination of two methods. first, QuEChERS fruit and vegetables extracts were injected in LC-MS/MS using a qualitative analysis of 300 targeted pesticides in the commercial MRM-triggered EPI screening method; and second, for presumptive positives, a second analysis was conducted using a quantitative/confirmatory MRM method we optimized for 55 previously detected and/or frequently used pesticides. The method combination and its limitations were critically discussed and it was tested on 200 fruit and vegetables samples from the Hungarian market.

Materials and methods

Reagents and materials

High-performance liquid chromatography (HPLC)-grade acetonitrile was purchased from Fisher Scientific (Loughborough, UK); deionized water was obtained from a Milli-Q reagent water system (Billerica, MA, USA); dimethyl formamide was obtained from Reanal (Budapest, Hungary); anhydrous magnesium sulfate was obtained from Scharlau Chemie S.A. (Sentmenat, Spain); and primary secondary amine (PSA)-bonded silica was obtained from Supelco (Bellefonte, PA, USA). Each sample was filtered through a 0.45-μm polyvinylidene difluoride (PVDF) filter (Roth, Carlsruhe, Germany) before injection. Acetic acid and sodium acetate (both Merck, Darmstadt, Germany) were used for the sample preparation procedure. Analytical-grade pesticide standards were ordered from Sigma-Aldrich (Steinheim, Germany), Dr. Ehrenstorfer GmbH (Augsburg, Germany) and Riedel-de Haen (Germany), and stored at −30°C. Individual standard stock solutions were prepared by dissolving the crystalline standards in acetonitrile (or dimethyl formamide for those insoluble in acetonitrile) to reach the final concentration of 1000–4000 $\mu g\,ml^{-1}$. For method optimization, individual standard solutions were used, which were prepared by diluting the stock solution to a concentration of 1–4 $\mu g\,ml^{-1}$. A standard mix solution in acetonitrile for preparation of calibration standards was prepared from the individual stock solutions to yield 10 $\mu g\,ml^{-1}$. All solutions were kept at −18°C before use.

Sample preparation

The acetate-buffered QuEChERS sample preparation method for pesticides (AOAC Official Method 2007.01) was applied to all the samples (Granby et al. 2004; Niessen et al. 2006; Kruve et al. 2008). After homogenization with a house-hold mill (equipped with stainless steel knives), a 15 g portion

of the homogenized sample was weighed into a 50 ml polytetrafluoroethylene (PTFE) tube and 100 µl of 50 µg ml^{-1} triphenyl phosphate (TPP) surrogate standard solution in acetonitrile was added followed by 15 ml of acetonitrile containing 1% acetic acid (v/v not accounting for purity). Then, 6 g $MgSO_4$ and 2.5 g sodium acetate trihydrate (equivalent to 1.5 g of anhydrous form) were added, and the sample was shaken forcefully for 4 min. The sample was then centrifuged (Hermle Z206A, Labcompare, San Francisco, CA, USA) at 4000 rpm (1860 rcf) for 5 min, and 5 ml of the supernatant were transferred to a 15 ml PTFE tube to which 750 mg $MgSO_4$ and 250 mg PSA were added. The extract was shaken using a vortex mixer for 20 s and centrifuged at 4000 rpm again for 5 min. Approximately 3 ml of the supernatant were filtered through a 0.45 µm PTFE filter (13 mm diameter), and 800 µl portions were transferred to autosampler vials. The extracts were evaporated to dryness under a stream of argon and reconstituted in 800 µl acetonitrile/water (20/80, v/v) for the LC-MS/MS analysis.

For the matrix-matched and standard addition calibrations, 4 × 80 µl of reconstituted samples were transferred into autosampler glass inserts, and 20 µl portions of 0, 250, 500 and 1250 ng ml^{-1} standard mix solutions containing the 55 pesticides in 25/75 acetonitrile/water (v/v) were added to reach the final additional concentrations of 0, 50, 100 and 250 ng g^{-1} equivalents, respectively.

LC-MS analysis

For LC analysis, an Agilent (Little Falls, DE, USA) 1100 HPLC system was used. It contained a binary pump, a degasser, column thermostat, and an autosampler. A reverse-phase C8 analytical column of 150 mm × 4.6 mm internal diameter (i.d.) and 5 µm particle size and a guard column of 12.5 mm × 4.6 mm and 5 µm particle size (both were Agilent Zorbax Eclipse XDB) were coupled to the LC system. Deionized water containing 0.1% formic acid (mobile phase component A) and acetonitrile (component B) were employed for the gradient programme, which started with 20% B for 3 min and was linearly increased to 100% B in 27 min (held for 3 min). The column was then re-equilibrated over 12 min back to 20% B. Thus, the total run time took 45 min. The flow rate was constant at 0.6 ml min^{-1}, and injection volume was 10 µl.

For the MS/MS analysis, an Applied Biosystems 3200 QTRAP system was used. It was equipped with a turbo ion-spray interface which was operated in positive mode (ESI+). Its parameters were as follows in the EPI screening approach for 300 pesticides and MRM quantitation for 55 pesticides, respectively: curtain gas flow = 20 and 10 psi; collision gas = 12 and 5 (arbitrary units); ion-spray voltage = 5000 and 5500 V; temperature = 450°C in both cases; ion source gas 1 flow = 40 and 50 psi, ion source gas 2 flow = 50 psi in both cases; and dwell time of one transition was 5 and 15 ms, respectively. Nitrogen was applied in the ion source and for the collision gas. In the screening method, vendor recommended declustering potentials of 20, 35, and 50 V were applied when the MRM signal automatically triggered the EPI process.

Applied Biosystems Analyst 1.4.2 software was used for instrument control and data processing. For the determination of 300 pesticides, the commercial method of Applied Biosystems (2005) and its library was used.

Results and discussion

Concept of qualitative screening and quantitation of pesticide residues

This study describes the combination of two parallel methods: (1) qualitative screening for 300 target pesticides by LC-MS/MS using MRM data-dependent triggered EPI mode with the commercial spectra database; and (2) confirmation and quantitative determination of the frequently used and/or previously detected pesticides using the MRM method we developed for 55 pesticides. Compared with possible alternatives available within our budget constraints, this concept was believed to give the widest scope with the least effort, and still give excellent qualitative and quantitative results, particularly when using QuEChERS for sample preparation. We sought to evaluate and implement the approach using commercial samples from the market. For efficiency and convenience, the two methods use the same instrument working with the same column and using the same gradient programme.

The workflow of the concept is shown in Figure 1, and the process is as follows: (1) conduct AOAC Official Method 2007.01 and analyse the extract using the EPI screening method for 300 pesticides; (2) if no pesticides are detected in the sample, no other measurements are necessary; (3) if any of the 55 pesticides from the developed MRM method are found to be positive in the EPI analysis, then re-inject the extract using the MRM method; (4) those presumptive positive residues are confirmed and quantified (or found not to be present) using reference standards and a method of standard additions; and (5) when a target pesticide is found to be positive in the EPI method but is not included among the 55 MRM analytes, then a reference standard of the pesticide is obtained, optimal ESI+ conditions are determined, and the MRM method is modified to include it for further qualitative

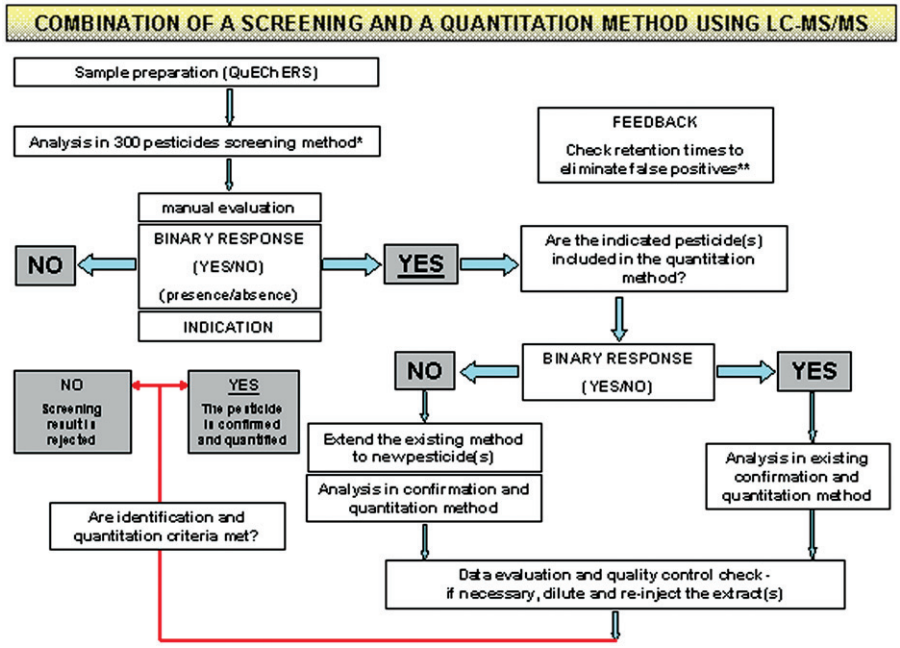

Figure 1. Workflow for the combination of a screening and quantitation approach by LC-MS/MS. *Commercial method of Applied Biosystems; **the retention times of the appropriate ion peaks have to be the same for the analyte in both methods.

and quantitative analysis. In this way, the MRM list of pesticide analytes grows over time.

Initial false-positive results from the screening method can be uncovered with this approach by comparing retention times between the methods, and findings that do not match are rejected. Furthermore, the peak shapes must be the same, the MS/MS product ion transitions must have similar ratios as the reference standards, the signal-to-noise (S/N) ratios for all ion peaks must be greater than 10, and quantitation must give a concentration greater than the reporting limit. Quantitation, identification, and confirmation of chemicals using MS techniques are discussed in the literature (Bethem et al. 2003; Milman 2005; European Commission 2007; Lehotay et al. 2008).

A major drawback in the use of matrix-matched calibration involves the need to find blanks for the matrices needed, thus we chose to apply a method of standard additions using multiple aliquots of the final sample extracts. This entailed taking three additional 80 µl aliquots of presumptive positive extracts and adding standards to achieve final additional equivalent concentrations 50, 100 and 250 ng g^{-1}, which were measured by the MRM method. We checked that the slopes of all detected pesticides were linear, which was nearly always the case for original sample concentrations less than about 200 ng g^{-1}, but if the slope was not linear for higher pesticide concentrations in the sample, we diluted the extracts five- or ten-fold to reach the linear region. After one year of routine application of this technique, we generated a calibration-slope database for the 55 investigated pesticides in twelve different fruit and vegetable matrices.

Screening identification of 300 pesticides in EPI

In the commercial EPI approach followed, a chosen (usually the most intensive) transition of each targeted analyte is monitored, which translates to 300 transitions in this method. If the intensity of the ion transition signal exceeds the chosen intensity threshold of 500 counts per second (cps), then the quasi-molecular ion is fragmented at 20, 35, and 50 V collision energies to yield product ions, which are further evaluated for possible positive findings (this technique is termed information dependent analysis by Applied Biosystems and data-dependent scanning or other terms by other vendors). The detection limits depended on the 500 cps threshold in our study, and naturally the system would be less sensitive if a higher threshold was used.

The total ion current (TIC) chromatogram consists of the intensities of all 300 monitored transitions plus any additional product ion signals that arise if the threshold was exceeded. Searches and presumptive identifications of pesticides from the TIC were started manually in this version of software (Analyst 1.4.2.). An advantage of the EPI approach is that MS/MS spectral library searching and comparisons are performed automatically by the software for the 20, 35, and 50 V collision energy spectra, but it allows checking and review by the analyst. Several criteria have to be met simultaneously for the presumptive identifications (also known as indications; Lehotay 2008) to be made: (1) a peak from the precursor and product ion transitions must all have the same retention time; (2) the characteristic fragmentation pattern of ions and their ratios obtained by the three different

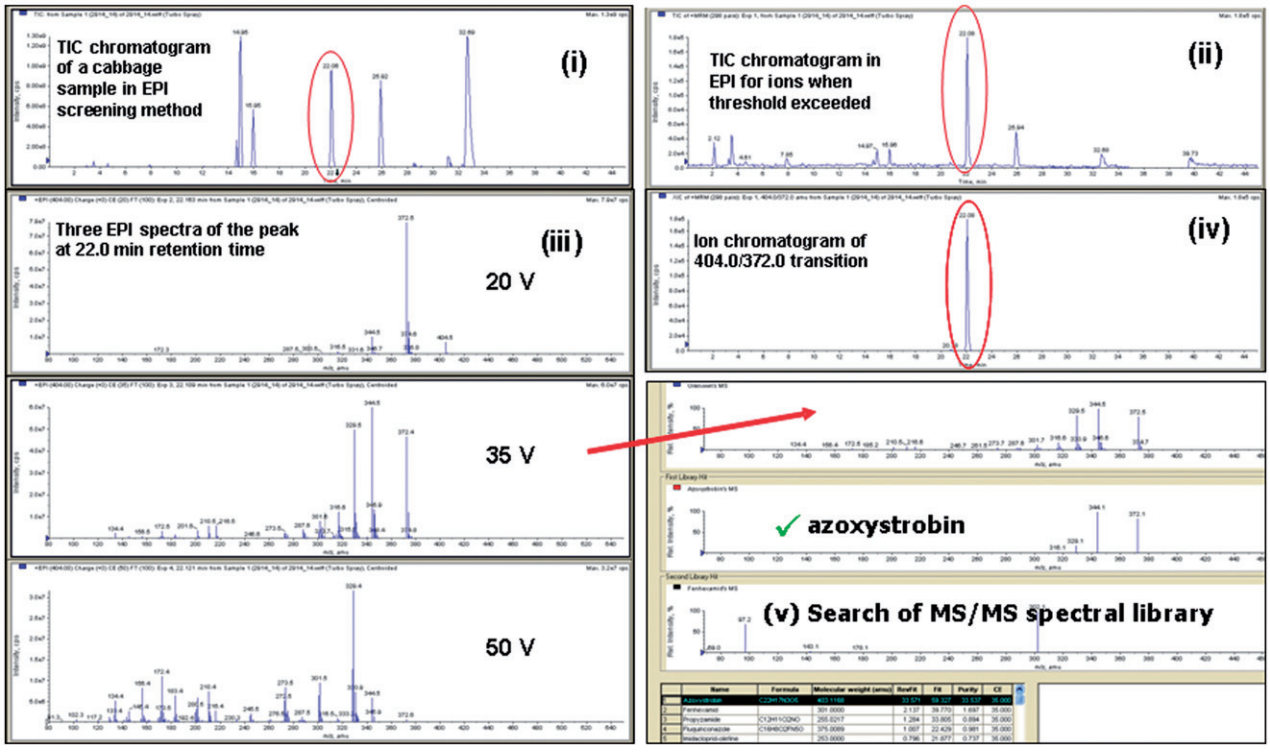

Figure 2. Example of the MRM-triggered EPI screening identification process: azoxystrobin was indicated in a cabbage extract at the retention time of 22.0 min using the pesticide MS/MS library of 300 pesticides.

collision energies have to match a compound in the library; and (3) the actual molecular weight of the pesticide presumptively identified has to correlate with the quasi-molecular ion of the precursor ion in EPI. This means that if the molecular weight of the pesticide in the library is [M], then the quasi-molecular ion in ESI+ must originate from $[M+H]^+$ $(M+1)$ or $[M+NH_4]^+$ $(M+18)$ in a few cases, such as carbamates in the spectral library. In practice, the hydrogen adduct $[M+H]^+$ occurs almost exclusively in this application.

Figure 2 shows an example for an EPI library-based identification of the pesticide azoxystrobin from a cabbage sample extract. This example shows the evaluation of a peak obtained at the retention time of 22.0 min. As observable in boxes (i) and (ii), the software detected an enhanced signal in the TIC that exceeded the intensity threshold of 500 cps, which triggered collision-activated dissociation of the most intense precursor ion at the given three collision energy voltages (default values of 20, 35, 50 V were used). As shown in box (iii), each averaged spectrum represent characteristic fragmentation patterns from the precursor ion. The m/z 404.5 ion that appears in the 20 V spectrum very likely indicates the quasi-molecular ion of the chemical of interest. The other two EPI spectra at increasing collision energies show how the m/z 404.5 ion disappears while ions of lower m/z (fragments of the probable quasi-molecular ion) increase. Box (iv) shows the extracted ion chromatogram for the m/z 404 to m/z 372 transition, which is the most intense and probably indicates the loss of a methoxy group from the molecule.

In this example in Figure 2, the MS/MS spectral library search of just the 35 V averaged spectrum is shown (box v). The software calculates the matching factors for the possible hits in the library and lists them on the screen. The findings can be sorted by name, formula, molecular weight, reverse fit, fit, purity, and collision energy. The collision energy is given along with the library spectra because different spectra are obtained at the different conditions. The software-calculated fit, reverse fit, and purity values are given in terms of percentage (fit and reverse fit relate to the spectral match of the sample spectrum to the library spectrum, and vice versa, respectively, and purity relates to the presence of extra ions in the sample spectrum that are not included in the library spectrum).

We analysed many different types of fruit and vegetable matrices in experiments, and found that the software worked well on its own to provide the top hit for pesticides in the library. The analyst needed only to review the retention times for the selected ion peaks for the indicated pesticide. In the example shown in Figure 2, azoxystrobin and its most intense first product ion transition, m/z 404 to m/z 372, is presented. The peak for that transition as shown in box (iv) gave a prominent Gaussian peak, and the analyst decided to reanalyse the extract in the MRM method, which included azoxystrobin.

False indications

False presumptive positives are when the pesticide is indicated to be present by the screening method (including analyst review), but is not detected by the MRM method. Many instances occurred when a positive precursor ion in the screening method would trigger the EPI function to occur, but the automated library search and analyst review of the spectra eliminated false presumptive positives. The EPI was 'falsely triggered' (threshold exceeded, but no pesticide was identified) most commonly when the TIC contained extensive chemical noise from the matrix background. These matrix components sometimes gave similar fragmentation patterns as pesticides in the library, such as a false indication of the mass spectrum for fenitrothion in lettuce extracts, but they were recognized and eliminated by the analyst with practice during manual evaluation. We deemed it better to re-inject the sample using the MRM method whenever we had reasonable doubt if a pesticide was present or not, but in practice there was very little reasonable doubt, and none of the re-injected samples from the EPI screening method gave false-positives in the MRM confirmation and quantitation method.

Possible interferences from matrix are known to increase with respect to lower masses (e.g., $m/z < 200$), and smaller pesticides tend to yield only a single product ion transition. Compounds with low mass or that yield only one fragment ion had greater potential for false-positives (and false-negatives) in general. An example of this is diphenylamine with MW = 169.2 amu, which gives only an m/z 170 to m/z 93.0 transition. In this case, the quasi-molecular ion disappears even using the lowest 20 V collision energy, which makes it difficult to detect in the EPI method. In EPI, if a pesticide is hidden behind a peak in the TIC, only one characteristic transition will be seen, but in the MRM method, a softer collision energy can be used to obtain two transitions.

False-negatives

Sometimes, when we analysed for the pesticides in the MRM method for the 55 pesticides, identifications would be made for pesticides that were not indicated in the EPI method for 300 pesticides. False-negatives mostly depended on the sensitivity difference between the two applied methods (see the limits of detection (LOD) in Table 1). The MRM method only found pesticides missed by the EPI screening method at low concentration levels (e.g., 10 ng g^{-1}). For example, boscalid in carrot (33 ng g^{-1}), in orange (17 ng g^{-1}), and in grapes (29 ng g^{-1}), quinoxyfen in grapes (10 and 12 ng ng^{-1}), and triadimenol in grapes (18 ng ng^{-1}) were detected solely by the MRM method. We do not know how many times false-negatives occurred when the extracts were not reanalysed by the MRM method, but the lack of glaring false-negatives for pesticides at high concentrations was a good sign that the EPI approach performed well.

However, the possibility of false-negatives increases if the threshold value (500 cps in this method) was enhanced, because it results in higher LODs in the EPI approach. We found this function to be critical; and 500 cps was optimal to obtain sufficiently low detection limits. A lower setting caused an increase in the number of peaks appearing in the TIC that created more manual work for the analyst. Naturally, new generation instruments with similar functions are more sensitive (and faster).

A likely reason for the lack of apparent false-positives was the manual evaluation of the TIC in the screening method, which required a high level of attention by the analyst to check for peaks for known ion transitions at known retention times for known pesticides. High background levels in some cases made this very difficult, especially for commodities with high fat (e.g., avocado) or high-flavour and -volatile (e.g., onion, garlic and ginger) composition.

Achievements and pitfalls

As in every method, this screening approach has advantages and disadvantages. One advantage is that pesticide reference standards are not required for all 300 targeted analytes in the library. Presumptive identifications are still possible for pesticides in the extracts (and potentially other chemicals that could be included in the MS/MS library). Extending the method to new analyte(s) is possible as new standards are obtained. Another advantage is that the method can screen for 300 pesticides, which is more than typical in monitoring methods, and extensive validation is not needed for pesticides that are not detected (albeit it is important to show that a pesticide analyte will be detected if it is present). In many cases, no pesticides are found in the fruit and vegetable samples, or only one or a few pesticides will be present. If the extract has few matrix components, then the manual evaluation of the given TIC can be conducted easily.

The main disadvantage with any targeted triple quadrupole method is that the list of analytes in the library is limited, and true unknown analysis cannot be conducted. The approach is blind to non-targets, unlike in the case of TOF techniques. Another disadvantage is that the screening method is time-consuming and can be problematic when the matrix is very complicated.

Another pitfall in the EPI screening method occurs when two target compounds co-elute. Only the most intense precursor ion will trigger the EPI process for that m/z, and the lower intensity ion, which may also exceed the 500 cps threshold, will not be detected.

Table 1. Detection parameters in the multiple reaction monitoring (MRM) quantitative method for 55 pesticides plus the triphenyl phosphate (TPP) surrogate standard, limit of detection (LOD) of the MRM and enhanced product ion (EPI) screening method.

Pesticide	MW (amu)	$[M+H]^+$ (m/z)	Product ions (m/z)	Ion ratio (%)	DP	EP	CEP	CE	CXP	t_R (min)	Peak width (min)	MRM LOD ($ng\,kg^{-1}$)	EPI LOD ($ng\,kg^{-1}$)
Acetamiprid	222.1	223.2	126.1	22	26	4.5	14	25	4	11.5	1.02	4	10
			56.1					33	4				
Azoxystrobin	403.4	404.0	372.0	33	31	5.5	18	19	12	22.0	1.03	1	4
			329.0					39	4				
Boscalid	343.2	343.0	307.0	61	56	8.5	16	25	10	22.3	0.81	4	40
			140.0					25	4				
Bupirimate	316.1	317.2	108.0	86	26	8.5	34	37	2	20.1	1.41	1	4
			166.2					31	4				
Buprofezin	305.4	306.0	201.0	93	31	4	16	17	4	25.7	1.56	1	10
			116.0					21	4				
Carbaryl	201.2	202.1	145.0	34	26	6	14	13	4	17.8	1.31	1	20
			127.0					35	4				
Carbendazim	191.2	192.2	160.0	17	36	8.5	12	23	4	3.6	1.45	1	4
			132.0					39	4				
Cyprodinil	225.1	226.2	76.9	80	41	4	16	55	4	19.4	1.69	4	10
			93.1					43	8				
Diazinon	304.3	305.0	169.0	62	36	5	16	31	4	26.5	1.29	1	1
			153.0					25	4				
Difenoconazole	406.3	406.1	251.0	32	41	6.5	30	31	4	24.9	1.06	1	10
			111.0					73	4				
Diflubenzuron	310.7	311.0	158.1	15	71	10.5	14	19	4	23.2	0.64	20	40
			141.1					39	4				
Dimethoate	229.3	230.0	125.0	97	16	4.5	14	25	4	11.3	1.01	1	20
			199.0					13	4				
Dimethomorph	387.0	388.2	301.2	58	51	4.5	20	27	4	19.5 and 20.0	1.60	1	40
			165.2					43	4				
Diphenylamine	169.2	170.0	93.0	17	51	8	12	31	4	24.3	1.23	<1	<1
			66.0					59	4				
Fenamiphos	303.4	304.0	217.0	59	41	4	14	29	4	21.7	1.25	1	4
			234.0					21	4				
Fenazaquin	306.4	307.0	57.0	80	41	4	14	39	4	29.4	1.62	1	10
			161.0					21	4				
Fenhexamid	302.0	302.1	97.1	63	51	4	16	33	4	22.2	1.28	1	20
			55.0					59	6				
Haloxyfop	361.0	362.1	316.1	[a]	61	7.5	16	23	8	23.5	0.78	10	
Hexythiazox	352.0	353.2	228.0	81	41	4.5	16	21	4	30.0	1.21	1	20
			168.1					33	4				
Imazalil	297.2	297.0	159.0	88	46	4	14	31	4	14.3	1.69	1	10
			201.0					23	4				
Imidacloprid	255.7	256.0	209.0	84	31	4.5	16	19	6	10.5	0.90	4	40
			175.0					23	4				
Indoxacarb	527.8	528.0	249.0	84	66	4	22	23	4	27.6	0.98	1	40
			150.0					31	4				
Isofenphos	345.4	346.0	217.0	34	16	2.5	14	29	6	28.1	0.62	1	40
			245.0					17	8				
Isofenphos-methyl	331.4	332.2	273.0	79	21	2.5	14	11	6	26.7	0.75	4	–
			231.0					17	8				
Kresoxim-methyl	313.4	314.0	206.0	59	21	9	14	11	4	25.4	1.14	20	40
			116.0					19	4				
Linuron	248.0	249.0	182.0	97	41	10	16	21	4	21.5	0.83	10	20
			160.0					25	4				
Malathion	330.4	331.1	127.0	86	26	8	16	17	4	24.1	0.84	1	40
			99.1					29	4				
Metalaxyl	279.3	280.0	220.0	85	31	4.5	14	17	4	18.1	1.09	1	10
			192.0					23	4				
Methamidophos	141.1	142.0	94.0	92	31	4.5	10	17	4	3.2	0.96	4	20
			125.0					17	4				

(*continued*)

Table 1. Continued.

Pesticide	MW (amu)	$[M+H]^+$ (m/z)	Product ions (m/z)	Ion ratio (%)	DP	EP	CEP	CE	CXP	t_R (min)	Peak width (min)	MRM LOD ($ng\,kg^{-1}$)	EPI LOD ($ng\,kg^{-1}$)
Methiocarb	225.3	226.0	169.0	73	26	9.5	14	13	4	21.1	1.20	1	–
			121.0					23	4				
Methiocarb-sulfone	257.3	258.1	122.1	69	36	5	16	21	4	12.7	0.98	–	–
			201.1					13	4				
Methiocarb-sulfoxide	241.3	242.2	185.0	35	31	4.5	14	17	4	8.4	1.37	4	20
			122.0					37	4				
Methomyl	162.2	163.1	106.0	94	21	4	10	13	4	6.5	1.64	4	40
			88.0					13	4				
Oxamyl	219.3	220.0	72.0	[b]	21	2	14	15	4	5.3	1.21	–	–
			90.0					13	4				
Penconazole	284.2	286.0	160.9	67	31	4.5	20	35	4	23.5	1.08	1	4
			70.0					31	4				
Pendimethalin	281.3	282.0	212.0	19	21	4	16	15	6	29.9	1.03	10	40
			194.0					23	4				
Phosalone	367.8	368.0	182.0	31	41	9.5	20	19	4	27.1	0.82	10	20
			111.0					53	4				
Phosmet	317.3	318.0	160.0	13	26	8.5	14	17	4	22.0	1.01	10	40
			133.0					49	4				
Pirimicarb	238.0	239.2	182.2	93	36	4.5	16	21	4	6.9	1.87	1	10
			72.0					31	4				
Pirimiphos-methyl	305.0	306.2	164.3	68	51	4.5	22	29	4	26.5	1.32	1	10
			108.1					41	4				
Prochloraz	376.7	378.1	310.1	37	21	4.5	24	17	6	20.2	1.16	1	20
			268.0					21	8				
Propamocarb	188.3	189.0	102.0	39	31	6	12	23	4	3.2	1.25	1	10
			144.0					17	4				
Propargite	350.5	368.2[c]	231.2	65	36	5.5	16	15	4	30.7	0.67	4	20
			175.3					19	4				
Pymetrozine	217.2	218.1	105.0	11	51	9	14	25	4	2.5	1.11	10	20
			78.1					55	4				
Pyraclostrobin	387.8	388.0	194.0	98	26	5	16	17	6	26.0	0.99	1	10
			163.0					29	4				
Pyrimethanil	199.3	200.2	107.1	62	46	7.5	12	31	4	15.9	1.47	1	10
			82.1					35	4				
Pyriproxyfen	321.4	322.0	96.0	62	31	2.5	20	21	4	28.9	1.48	1	4
			185.0					29	4				
Quinoxyfen	308.1	308.0	197.0	97	61	4	14	43	4	27.3	1.26	1	20
			162.0					61	4				
Tebuconazole	307.8	308.1	70.0	55	41	4.5	14	41	4	22.6	1.26	1	10
			125.0					45	4				
Thiabendazole	201.3	202.0	175.0	84	56	6.5	14	35	4	3.8	1.66	1	4
			131.0					43	4				
Thiacloprid	252.7	253.0	126.0	18	51	4	16	27	4	13.6	1.55	4	20
			99.0					57	4				
Thiamethoxam	291.0	292.1	211.2	39	26	9.5	16	17	4	7.9	1.23	10	40
			181.1					29	4				
Thiophanate-methyl	342.4	343.1	151.1	14	26	6.5	18	25	4	15.8	1.04	4	20
			311.1					17	10				
TPP	326.0	327.0	152.0	78	61	12		43	4	25.9	1.07	1	–
			215.0					33	4				
Triadimenol	295.8	296.0	70.0	9	21	4	14	21	4	20.3 and 20.8	1.76	10	40
			227.0					13	4				
Trifloxystrobin	408.4	409.2	186.1	37	26	7	18	23	4	27.9	1.25	1	4
			206.2					19	4				

Notes: [a]No second ion.
[b]Insensitive signal.
[c]$[M+NH_4]^+$ $(M+18)$.
DP, declustering potential; EP, entrance potential; CEP, collision cell entrance potential; CE, collision energy; CXP, collision cell exit potential.

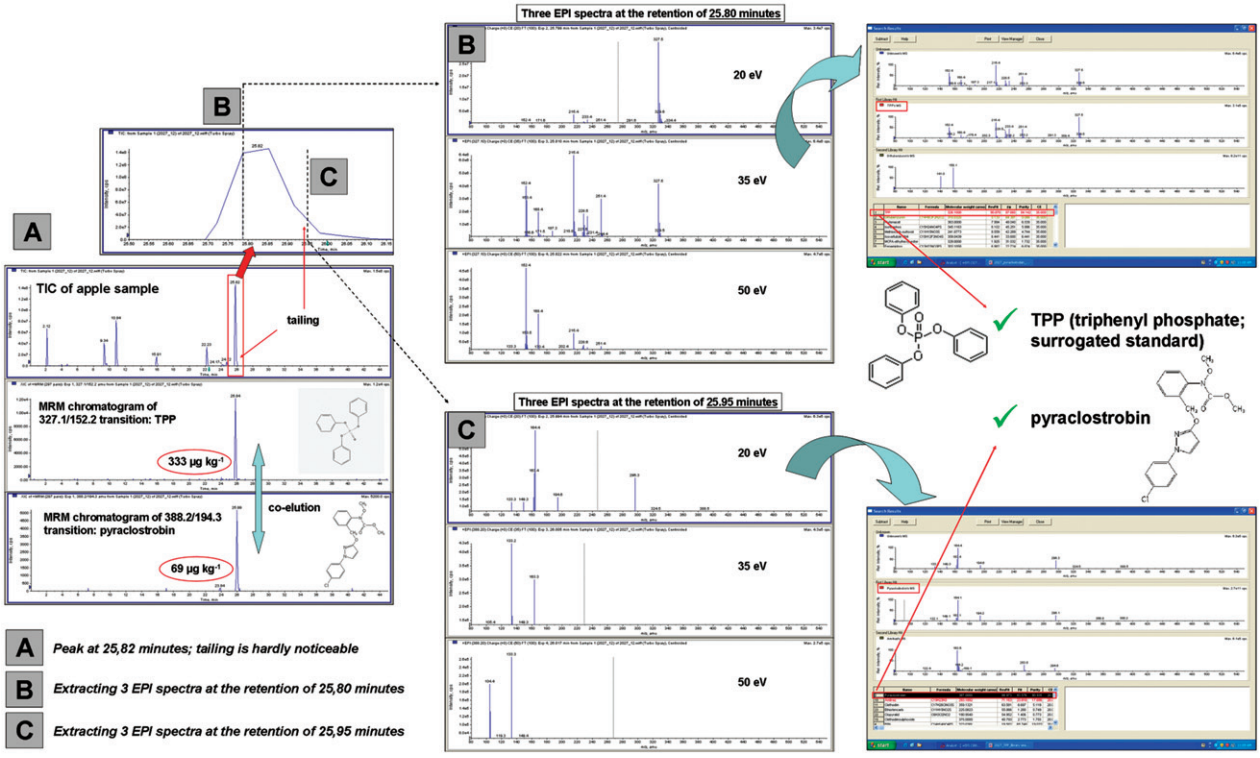

Figure 3. Careful data review was needed due to co-elution of TPP and pyraclostrobin: a) TIC and the MRM chromatograms of TPP and pyraclostrobin; b) EPI spectra extracted from the peak apex (25.80 min) and identification of TPP (added at 333 mg kg^{-1} into the sample); and c) EPI spectra extracted from the tail of the peak (25.95 min) and identification of pyraclostrobin.

This can also occur if an intense matrix peak at a different m/z masks the precursor ion of a pesticide analyte. The best example of analyte–analyte co-elution is TPP ($t_R = 25.8$ min) and pyraclostrobin ($t_R = 26.0$ min), which is presented for apple in Figure 3. Pyraclostrobin is hardly visible in the TIC because it is masked behind the large TPP peak. A small tailing effect can be seen in the enlarged picture of the TPP peak. The three EPI spectra at 25.8 min show a clear fragmentation pattern, which is known to correspond to the TPP added in all samples at 0.333 mg kg^{-1} during extraction. Of course, the identification of TPP is expected (required for quality control purposes) in all samples, but the software cannot find pyraclostrobin at low concentrations appearing 0.15 min later than the TPP peak apex, despite the different fragmentation ions (327.1/152.2 for TPP versus 388.2/194.3 for pyraclostrobin). The EPI function only fragments the quasi molecular ion of the more intensive transition, thus, the transition for pyraclostrobin's fragmentation pattern was not recorded in this case. The analyst noticed that pyraclostrobin also appeared in the apple sample extract, which was confirmed by the MRM method and determined to have a concentration of 0.069 mg kg^{-1}. Due to this 'pitfall', the analyst must take extra care to review the EPI screening chromatograms for the presence of pyraclostrobin near the TPP peak in all samples.

Confirmation of frequently detected pesticides in MRM mode

Confirmation

For confirmation and quantitation purposes, the two most intensive transitions for the 55 more commonly detected pesticide analytes in the MRM method are monitored. The optimized conditions were entered into the method file as given in Table 1 (only one transition could be obtained in the case of haloxyfop), which totalled 111 transitions. Table 1 also lists retention times, peak widths, ion ratios, and LODs for the analytes. The quasi-molecular ions were $[M+H]^+$ $(M+1)$ in all cases except for propargite, which had a strong ammonium adduct $[M+NH_4]^+$ $(M+18)$.

Achievements and pitfalls

The MRM confirmation/quantitation method targeted those residues known to have occurred in Hungarian and secondarily in European markets (data pool of the Community Reference Laboratories for Residues of Pesticides; see http://www.crl-pesticides.eu/), and consequently these are targeted from the aspect of their frequent occurrences. It was not worth the effort to include all 300 analytes in the MRM method and QuEChERS has already been extensively validated in many laboratories. Thus, we chose the 55 most prominent pesticides, which were those that had been

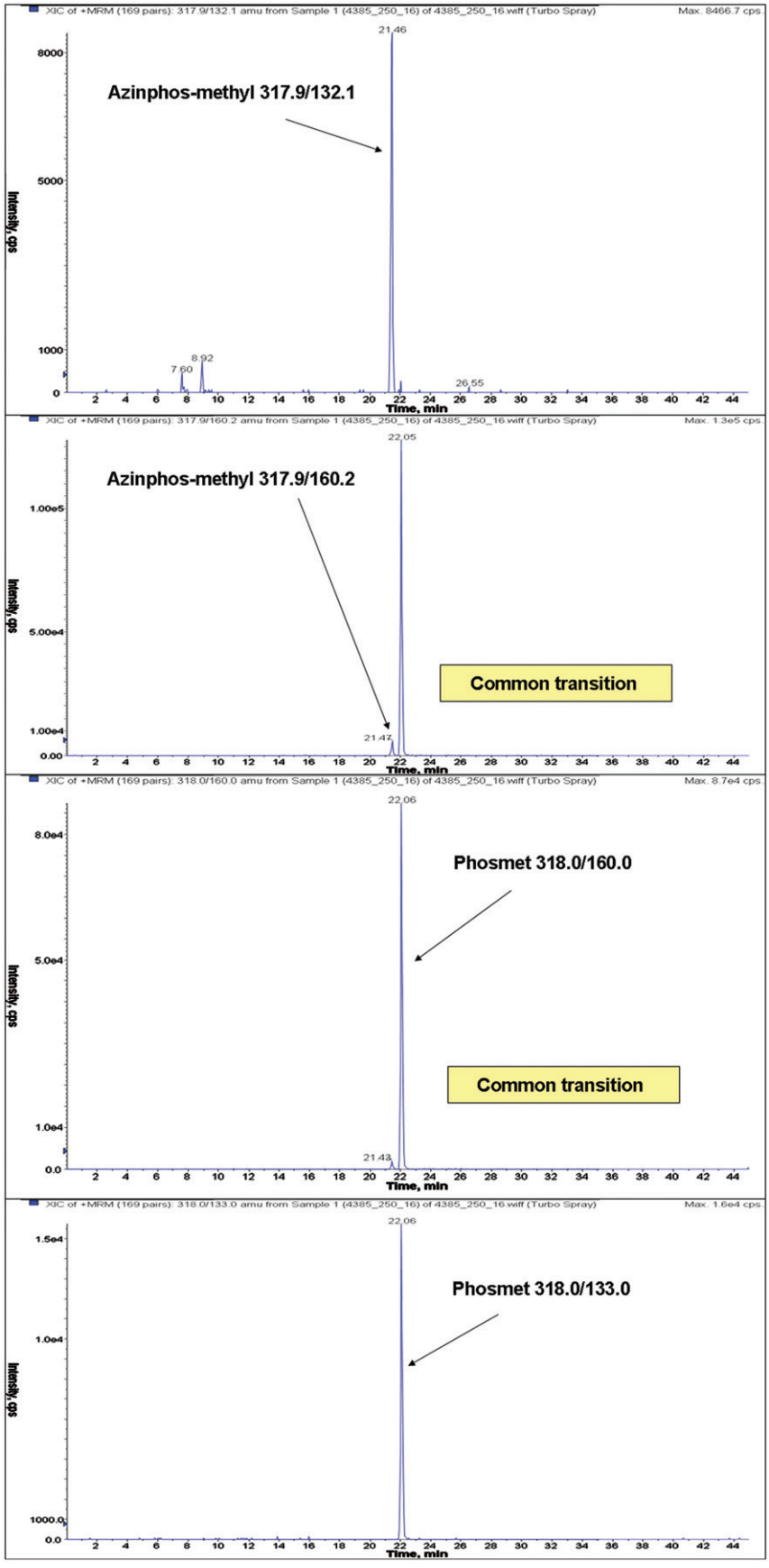

Figure 4. Situation for similar ion transitions and retention times of azinphos-methyl and phosmet.

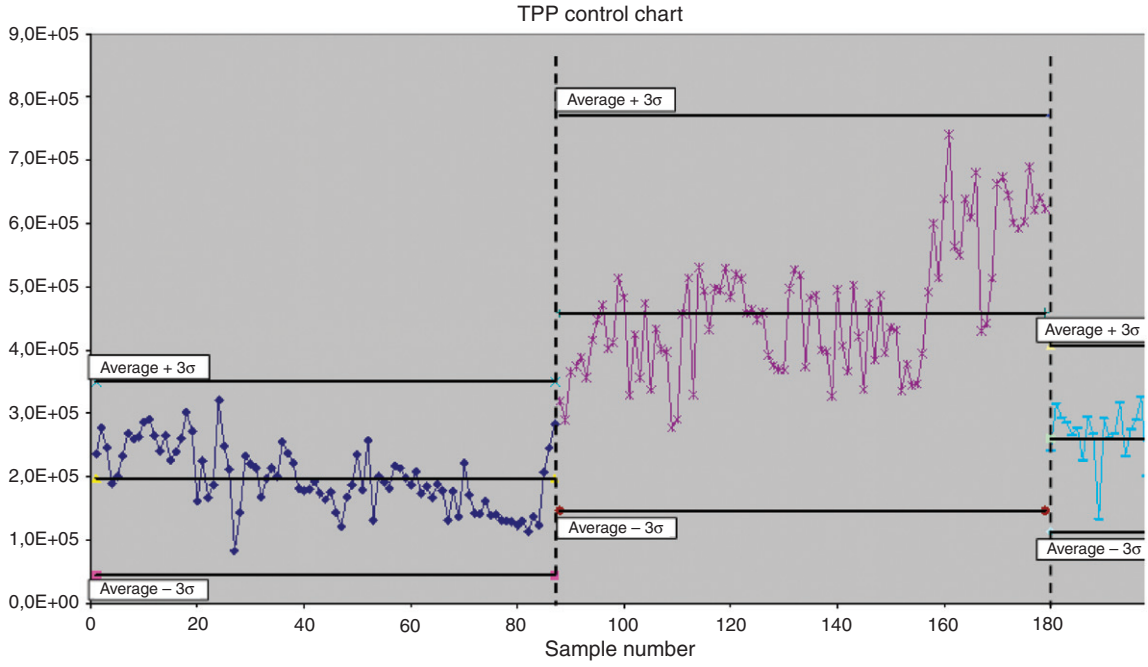

Figure 5. Quality control chart of 0.333 mg kg^{-1} equivalent concentration of triphenyl phosphate (TPP) as the surrogate standard in 3 groups of results for the 200 tested samples.

previously detected in the monitoring programme. The selectivity and sensitivity of the MRM method are better than the EPI method, as the LODs in Table 1 demonstrate (LODs of the 245 other compounds in EPI were not measured, but they are believed to be within the range of the 55 shown). The LODs were estimated by injecting 1, 4, 10, 20 and 40 ng g^{-1}-equivalent standard mix solutions (not sample extracts), and when both transitions for an analyte in the MRM method gave an observable signal peak at the correct t_R, then that concentration was assigned to be the LOD. In the EPI screening method, the LOD estimation required preparation of five mixture solutions eleven pesticides each, which were chosen to avoid co-elutions. The lowest concentration at the pesticide could be identified by the screening method was recorded as the LOD.

The reported LODs are well below the MRLs for those pesticide that are registered in typical fruits and vegetables, and the 0.01 mg kg^{-1} level is often reached for those pesticides that are not registered in the commodities analysed. It is not likely that false-negatives above the reporting limits create a problem in our monitoring approach for LC-amenable pesticides, particularly for those pesticides with MRLs.

Matrix interferences are difficult to predict, but the analyst can be made aware of possible analyte–analyte interfering transitions at similar t_R. For example, the potential interfering transition of azinphos-methyl and phosmet is presented in Figure 4. Whereas the 317.9/160.2 and the 318.0/160.0 transitions can interfere with each other, their identification in the MRM method relies also on their slightly different t_R and second-ion transitions. To meet identification criteria, the ion ratios for the pesticide transitions must fall within the intervals listed in the laboratory quality control guidelines in the European SANCO directive (European Commission 2007).

Matrix effects and calibration slope database

The weakness of LC-MS/MS for quantitative purposes are matrix effects, typically from ion suppression in ESI mode. Matrix effects lead to uncertainty in the accuracy of the quantitative results, and matrix-matched calibration is the most common approach used to account for matrix effects. The need for blanks and extra extractions required for matrix-matching is not convenient and there are no guarantees that the matrix compensation is the same between the different samples. When multiple matrix types appear in the same sequence, it is questionable if one matrix can be used to represent another (the practice is questionable even within the same matrix type). However, matrix-matching is felt to be more practical compared with the few alternatives in routine multiclass, multiresidue analysis, and matrix-matching has become an established custom (Kmellár et al. 2008). Indeed, results from proficiency testing in pesticide analysis generally demonstrates that matrix-matching gives acceptable accuracy.

Table 2. Average least linear-squared calibration line slopes for the pesticide analytes (listed in order of increasing t_R) normalized to the equivalent 0.333 mg kg^{-1} triphenyl phosphate (TPP) internal standard signal (*10^{-3}) in matrix-matched calibration standards for twelve different matrices ($n = 10$).

Pesticide	Commodity												Overall average ± SD ($n = 12$)
	1	2	3	4	5	6	7	8	9	10	11	12	
Pymetrozine	8.2	15.7	**8.3**	9.4	12.1	**8.0**	**11.5**	6.0	8.8	12.2	8.1	**7.3**	**9.6** ± 2.6
Methamidophos	**4.0**	4.1	3.8	**4.5**	3.3	**4.9**	**4.1**	**3.6**	**3.3**	**4.1**	3.4	3.1	3.8 ± 0.5
Propamocarb	13.6	15.4	13.6	14.0	14.3	**15.6**	**15.0**	**14.7**	**12.2**	13.8	12.6	12.7	14.0 ± 1.0
Carbendazim	16.7	18.5	**17.1**	**17.2**	16.8	**19.9**	**17.3**	**15.1**	**14.9**	16.3	15.2	**15.3**	16.7 ± 1.4
Thiabendazole	7.6	**8.7**	7.8	**9.1**	8.5	**8.6**	**7.9**	**7.7**	**6.6**	6.9	6.7	**6.7**	7.7 ± 0.8
Methomyl	5.2	5.5	**5.2**	**4.6**	5.1	**6.5**	5.6	4.4	4.4	4.9	4.8	4.7	5.1 ± 0.6
Pirimicarb	**14.2**	14.8	**13.1**	**16.1**	13.4	**17.1**	**15.5**	**13.4**	**12.6**	**14.0**	11.0	11.9	13.9 ± 1.7
Thiamethoxam	1.7	1.9	1.7	1.4	1.8	**2.0**	**1.6**	**1.4**	1.5	1.8	1.5	**1.4**	1.6 ± 0.2
Methiocarb-sulfoxide	**7.0**	**7.8**	**8.4**	**8.5**	13.6	**11.6**	**9.8**	**7.0**	**8.2**	**10.9**	**9.0**	**9.6**	9.3 ± 1.9
Imidacloprid	1.9	**2.5**	**2.3**	1.7	**2.6**	**2.6**	**2.2**	1.8	1.7	2.0	2.2	**2.1**	2.1 ± 0.3
Dimethoate	6.5	7.1	**6.6**	6.4	6.6	**8.5**	7.0	5.8	5.4	5.8	5.3	6.5	6.5 ± 0.8
Acetamiprid	6.4	**7.4**	7.1	6.9	**7.6**	**8.1**	**6.9**	6.1	**5.6**	6.1	6.0	**6.3**	6.7 ± 0.7
Methiocarb-sulfone	**0.9**	**1.6**	**1.2**	**1.7**	**5.3**	**1.9**	**2.9**	**1.8**	**1.5**	**2.2**	**2.0**	**2.9**	**2.2** ± 1.1
Thiacloprid	**8.6**	**10.5**	**9.8**	9.2	**11.2**	**10.8**	**8.6**	**7.6**	**7.0**	8.1	8.9	**9.6**	9.2 ± 1.2
Imazalil	**3.9**	4.4	3.7	**4.2**	2.9	**4.7**	**4.4**	4.3	**3.4**	**3.8**	3.2	3.3	3.8 ± 0.5
Thiophanate-methyl	8.2	**8.6**	**7.4**	7.3	8.5	**8.7**	**8.9**	**7.3**	**6.7**	**7.6**	**7.4**	**7.9**	7.9 ± 0.7
Pyrimethanil	6.6	7.6	6.5	**7.7**	7.1	**7.8**	7.0	**7.1**	**5.9**	6.0	5.8	6.0	6.8 ± 0.7
Carbaryl	14.3	**15.6**	14.7	**12.0**	**17.7**	**18.1**	**15.8**	10.6	**12.4**	13.3	11.4	15.2	14.3 ± 2.3
Metalaxyl	15.1	**17.7**	16.0	16.4	**16.3**	18.5	17.2	17.4	13.0	13.7	14.1	16.1	16.0 ± 1.6
Cyprodinil	**3.7**	3.8	**3.3**	**3.9**	3.1	**3.5**	**4.1**	**3.6**	**3.4**	**3.7**	2.8	3.1	3.5 ± 0.4
Dimethomorph	4.9	5.6	5.3	4.9	**5.9**	6.1	5.5	5.5	4.3	4.7	5.0	5.4	5.3 ± 0.5
Bupirimate	**4.9**	**4.9**	**4.2**	**5.2**	4.1	**5.4**	**5.3**	**5.3**	**4.4**	**4.5**	3.8	4.2	4.7 ± 0.5
Prochloraz	6.1	7.0	**5.8**	5.5	4.5	**7.4**	7.0	5.3	**5.6**	5.6	5.7	6.1	6.0 ± 0.8
Triadimenol	**7.2**	**8.4**	**6.5**	**7.1**	9.5	**11.0**	**9.3**	**7.7**	**6.6**	**7.8**	**6.1**	**7.2**	7.9 ± 1.4
Methiocarb	8.8	**10.5**	9.9	**7.9**	11.3	**11.8**	10.3	**9.0**	**8.3**	8.8	9.6	10.5	9.7 ± 1.2
Linuron	2.8	4.1	2.7	2.8	3.1	2.9	2.9	3.0	2.0	3.1	2.7	2.8	2.9 ± 0.4
Fenamiphos	**16.5**	19.0	16.8	16.9	**16.8**	19.3	**18.6**	18.5	**14.0**	**16.1**	14.5	17.2	17.0 ± 1.6
Azoxystrobin	17.4	18.2	16.9	**16.2**	17.7	**19.7**	19.4	**17.9**	15.8	16.4	16.2	19.2	17.6 ± 1.3
Phosmet	**4.7**	**5.3**	**5.5**	**4.0**	**7.3**	**7.2**	**6.6**	**3.2**	**4.9**	6.1	5.8	**6.2**	**5.6** ± 1.2
Fenhexamid	4.8	5.9	5.4	5.0	5.3	**5.8**	5.8	4.9	**4.3**	5.2	5.0	5.1	5.2 ± 0.4
Boscalid	2.5	**3.0**	2.9	**2.4**	3.1	**3.0**	3.0	2.6	**2.2**	2.6	2.6	2.9	2.7 ± 0.3
Tebuconazole	**14.8**	18.3	**15.9**	**13.5**	16.1	**18.0**	**17.9**	**15.8**	**14.1**	**15.6**	14.5	14.5	15.8 ± 1.5
Diflubenzuron	2.4	**3.7**	2.5	2.2	**3.5**	**2.9**	2.6	**2.7**	1.9	2.2	2.1	2.4	2.6 ± 0.5
Haloxyfop	1.0	2.4	**1.2**	1.1	2.5	**2.8**	**1.0**	**1.3**	0.8	**1.1**	1.2	**1.0**	**1.4** ± 0.7
Penconazole	4.3	8.6	**4.9**	4.4	7.7	9.2	4.4	**5.4**	3.3	4.2	4.8	4.0	**5.4** ± 1.9
Malathion	8.0	**8.7**	8.3	**7.4**	9.7	**9.8**	**8.9**	**7.5**	**7.3**	7.4	7.8	8.9	8.3 ± 0.8
Diphenylamine	**9.1**	11.2	**9.8**	**8.8**	9.8	11.4	**11.0**	**9.2**	**8.3**	10.0	9.3	**8.4**	9.7 ± 1.0
Difenoconazole	**11.6**	13.1	**11.2**	**11.3**	13.2	**14.2**	**13.9**	**10.6**	**11.4**	11.5	11.6	**12.0**	12.1 ± 1.1
Kresoxim-methyl	**0.2**	**0.3**	**0.2**	**0.3**	**0.5**	**0.4**	**0.2**	**0.3**	**0.2**	**0.2**	**0.2**	**0.2**	**0.3** ± 0.1
Buprofezin	**27.4**	**28.6**	24.1	26.9	26.7	**26.4**	**31.7**	**27.7**	**25.5**	**24.9**	**22.2**	25.5	26.5 ± 2.3
Pyraclostrobin	11.3	11.2	10.7	**10.0**	10.9	12.3	12.2	**10.6**	10.2	10.0	10.0	11.5	10.9 ± 0.8
Diazinon	**27.6**	**30.7**	**25.2**	**31.6**	24.4	**31.3**	**32.6**	**31.7**	**26.7**	**28.2**	**22.3**	**23.6**	28.0 ± 3.4
Pirimiphos-methyl	**19.6**	20.9	**17.4**	**21.6**	18.3	**22.3**	**22.6**	**20.4**	**18.7**	**19.9**	**16.1**	16.7	19.5 ± 2.0
Isofenphos-methyl	**0.8**	**1.1**	**0.4**	**1.8**	**1.5**	**1.9**	**0.8**	**1.4**	**0.8**	**0.7**	**0.7**	**0.8**	**1.1** ± 0.5
Phosalone	3.2	4.2	2.9	3.4	**3.2**	2.5	3.4	3.1	**2.3**	**3.8**	**3.4**	2.4	3.2 ± 0.5
Quinoxyfen	**5.3**	**4.8**	**4.4**	**4.3**	4.2	**4.3**	**5.8**	**4.1**	**4.7**	**5.2**	**4.1**	**4.3**	4.6 ± 0.5
Indoxacarb	0.9	**1.0**	0.9	**0.8**	1.0	**1.0**	**1.1**	**0.8**	**0.8**	1.0	0.9	**1.1**	0.9 ± 0.1
Trifloxystrobin	15.0	**12.5**	12.2	14.8	**11.6**	**13.8**	16.0	**13.0**	**12.9**	13.2	**11.4**	13.4	13.3 ± 1.3
Isofenphos	**0.4**	**0.5**	**0.2**	**0.7**	**0.6**	**0.8**	**0.4**	**0.9**	**0.5**	**0.2**	**0.3**	**0.3**	**0.5** ± 0.2
Pyriproxyfen	**26.2**	**20.4**	**19.6**	22.9	**21.1**	**20.0**	**27.9**	**20.1**	**22.9**	**26.6**	**17.8**	21.5	22.2 ± 3.0
Fenazaquin	**16.7**	**13.3**	**11.7**	15.8	**11.8**	**12.7**	**16.9**	**13.4**	**15.4**	**16.2**	**11.0**	**11.8**	13.9 ± 2.1
Pendimethalin	**2.4**	**1.8**	**1.8**	**2.0**	**1.8**	**1.8**	**2.7**	**1.8**	**1.9**	**2.2**	**1.8**	2.3	2.0 ± 0.3
Hexythiazox	**2.7**	**1.8**	**2.0**	**2.4**	**1.8**	**1.8**	**2.7**	**1.7**	**2.0**	**2.3**	**1.9**	2.2	2.1 ± 0.3
Propargite	**0.8**	**0.3**	**0.5**	**1.0**	**0.4**	**0.4**	**0.8**	**0.7**	**0.6**	**0.5**	**0.3**	**0.7**	**0.6** ± 0.2

Notes: 1, Tomato; 2, apple; 3, lettuce; 4, cucumber; 5, carrot; 6, mushroom; 7, grape; 8, lemon; 9, pepper; 10, pear; 11, potato; and 12, cabbage. Values shown in bold indicate a relative standard deviation (RSD) >20%.
SD, standard deviation.

The method of standard addition calibration is another alternative, but this also has can be questioned due to potential non-linearities in the calibration range of the analysis. We chose to spare ourselves the time and materials needed for additional sample preparation of blanks for matrix-matching by taking additional aliquots of each presumptive positive extract for the method of standard additions. All 55 pesticides were added to each extract for confirmation and quantitation in the MRM method even when only a single pesticide was indicated in the EPI screening method. In this way, we generated a large amount of data for calibration experiments and comparisons.

At first, we constructed a database about the obtained calibration slopes originating from twelve selected matrices. A total of ten calibrations were averaged per each pesticide in each matrix, which were performed in the analyses of the 200 samples over the course of a year. The comparison of slopes over time showed a high variation for many pesticides even for the same matrix when the signals were not normalized to the surrogate standard, TPP. To track method performance, we constructed a control chart of peak areas of TPP, which was added at $0.333\,mg\,kg^{-1}$ to all samples. As shown in Figure 5, a recognizable change occurred after 90 samples when the source was thoroughly cleaned and detector voltage changed from 2400 to 2500 V (maximum = 3200 V). The curtain plate of the ion source was usually cleaned once per week, but at this time, we also cleaned the orifice plate and the Q0. Considering that the fluctuations of TPP intensities included sample preparation as well as instrument performance aspects, the TPP signal fluctuations were reasonably small. Thus, both the sample preparation and the MRM method were performing quite well, especially considering that so many samples and sample types were analysed.

By normalizing the peak areas of the pesticides to peak areas of TPP for each calibration point, the consistency of day-to-day calibration slopes improved. Table 2 presents the TPP-normalized matrix-matched calibration slopes for the twelve different matrices tested. The 54 pesticides (oxamyl was too insensitive for inclusion) are listed in order of t_R possibly to infer large matrix co-eluting peaks that induced ion suppression in that part of the chromatogram (of course, TPP could also be affected by matrix co-elutants, which complicates interpretation of the results). The values shown in bold designate average slopes when relative standard deviation (RSD) $>20\%$. Many of the pesticides gave very consistent slopes from day-to-day and matrix-to-matrix, as observable by the overall average slope $\pm$ standard deviation (SD) column in Table 2 among the twelve matrices. Those pesticides with consistent slopes likely have little or no matrix effects in the different matrices. Conversely, some pesticides gave highly variable slopes in all matrices, which indicated matrix effects or, more likely and simply, that the pesticide is less stable or has more problematic analysis. This is expected in routine multiclass, multiresidue analysis, especially over the course of one year at different instrument maintenance conditions. Even for those pesticides with variable slopes within a particular matrix, only a few pesticides gave overall average slopes with $>20\%$ RSD (shown in bold) among the twelve matrices tested.

Table 3. Distribution of the total and samples found to contain residues per commodity analysed.

	Commodity	Number positive/total samples
Fruits	Apple	7/14
	Grape	6/9
	Lemon	8/8
	Watermelon	2/8
	Pear	5/6
	Peach	3/6
	Banana	5/5
	Nectarine	3/3
	Pineapple	0/2
	Strawberry	2/2
	Grapefruit	2/2
	Orange	2/2
	Melon	0/2
Vegetables	Pepper	10/23
	Lettuce	10/18
	Tomato	5/15
	Cabbage	7/16
	Carrot	5/6
	Garlic	2/5
	Cucumber	4/5
	Parsnip	1/5
	Onion	0/5
	Celery	2/3
	Radish	0/2
	Pumpkin	0/2
Others	Mushroom	3/8
	Miscellaneous	6/9
Total		100/200

Results of a one-year routine application

During a one-year routine application of this monitoring approach (March 2008–February 2009), 200 samples from the Hungarian market were analysed (15–20 samples/month). In all 13 types of fruit, 14 types of vegetables, and ten other sample types were analysed, as listed in Table 3. The confirmed positive results sorted by pesticides and their concentration range in the samples are presented in Table 4. Vegetable samples originated mainly from Hungary and nearby European Union countries, but a large number of fruit samples, especially tropical fruits, were imported from southern European countries

Table 4. Determined pesticides and their concentration ranges in the 200 samples analysed.

Pesticide	Fruits (71 samples)			Vegetables/other (129 samples)		
	Number of positive samples	Minimum–maximum ($mg\,kg^{-1}$)	Number of violations	Number of positive samples	Minimum–maximum ($mg\,kg^{-1}$)	Number of violations
Fungicides						
Azoxystrobin	1	0.109		14	0.010–0.551	
Boscalid	2	0.017–0.029		1	10.4	1
Bupirimate	2	0.005–0.008		–	–	
Carbendazim	4	0.041–0.064		4	0.041–0.352	2
Cyprodinil	7	0.010–0.364		–	–	
Diethofencarb	–	–		1	0.030	
Difenoconazole	1	0.025		–	–	
Dimethomorph	–	–		1	0.176	1
Diphenylamine	16	0.007–0.081	1	29	0.006–0.202	4
Fenhexamid	1	0.189		–	–	
Imazalil	15	0.030–6.655		–	–	
Metalaxyl	2	0.087–0.111		2	0.032–0.060	
Myclobutanyl	1	0.102		–	–	
Prochloraz	3	1.045–5.043		–	–	
Propamocarb	–	–		5	0.100–0.475	
Pyraclostrobin	1	0.069		1	0.230	
Pyrimethanil	2	0.010–0.039		–	–	
Quinoxyfen	2	0.010–0.012		–	–	
Spiroxamine	1	0.189		–	–	
Tebuconazole	–	–		1	0.040	
Thiabendazole	10	0.035–0.899		–	–	
Triadimenol	1	0.018		–	–	
Herbicides						
Haloxyfop	–	–		1	0.637	1
Linuron	–	–		1	0.314	
Insecticides						
Acetamiprid	1	0.035		3	0.037–0.098	
Buprofezin	–	–		1	0.043	
Diflubenzuron	4	0.020–0.127		–	–	
Diazinon	2	0.020–0.029	1	–	–	
Dimethoate	–	–		1	0.244	1
Indoxacarb	–	–		1	0.054	
Phosmet	2	0.370–0.447		–	–	
Pirimicarb	2	0.022–0.024		–	–	
Pymetrozine	–	–		1	0.199	
Pyriproxyfen	–	–		1	0.010	
Thiamethoxam	–	–		2	0.111–0.290	

(e.g., Spain, Italy, and Greece) or non-European Union countries in Central or South America, Africa, and Turkey. Exactly half of the samples gave positive confirmations for at least one residue (up to five pesticides were found in one sample), and none of the 300 LC-amenable pesticides was detected in the other half.

The most frequently detected pesticides were the fungicides imazalil and thiabendazole in fruits and azoxystrobin in vegetables. Diphenylamine was detected in the samples independently from the commodity (we checked the tubes, acetonitrile, water and other reagents for contamination of diphenylamine, but found that everything was clear). Among these, the determined concentrations of azoxystrobin were relatively low ($0.01–0.55\,mg\,kg^{-1}$), while thiabendazole and imazalil often were found at levels as high as 0.90 and $6.66\,mg\,kg^{-1}$, respectively. However, none of these results exceeded the current European Union MRLs. Prochloraz was found at high concentration in three lemon samples (from Argentina and Turkey) and nicobifen was found to occur at the MRL ($10\,\mu g\,kg^{-1}$) in a cabbage sample from Hungary.

Diphenylamine mostly appeared in samples during the summer months at low concentrations ($0.006–0.202\,mg\,kg^{-1}$), and the appearance was independent from the country of origin and the commodity. Besides being a fungicide, diphenylamine is widely used as a rubber antioxidant and accelerator, solid-fuel rocket propellant, stabilizer for explosives, for preparation of pharmaceutical and veterinary medicine, as a storage preservation of apples, and as a reagent in

analytical chemistry. It is conceivable that the stored fruits and vegetables were treated with diphenylamine to preserve their quality and freshness, or that it was a contaminant that did not originate from a pesticide application.

Conclusions

The developed combination of the two described methods permitted the fast and easy qualitative screening of 300 target pesticides in a 45-min LC-MS/MS run. Although the manual evaluation of the given chromatograms increased the analysis time by an additional 10 min per sample, very little time, cost, and labour was spent on sample preparation. In the case of dirty samples, some false indications were observed, but these were caught by the use of the MRM confirmatory and quantitative method for the 55 more common pesticides. The construction of standard addition calibration standards was carried out with the same extract that was previously injected for screening the 300 compounds. A large calibration database in different matrices was collected to show the consistency of the average calibration slope, which helped us check the accuracy of the calculated results from the method of standard additions.

The one-year routine application of this scheme was a comprehensive study that showed consumer exposure of pesticides from fruits and vegetables that can be purchased in the Hungarian market. Carbendazim, dimethomorph, diphenylamine, nicobifen, haloxyfop, diazinon and dimethoate exceeded the current European Union MRLs a total of twelve times among the 200 samples analysed.

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